

Host Growth Rate Affects Bacteriophage Lambda Burst Size

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ABSTRACT

Life history traits such as growth rate and burst size involve the multiplication of phages in the host bacterial cell as a means of transmission and survival. As a consequence phage fitness depends on the physiological state of the host based on nutrient availability. In this study I tried to elucidate the effect of *E. coli* growth rate on burst size in phage lambda. It was determined that burst size increases linearly from 11 PFU·cell⁻¹ to 147 PFU·cell⁻¹ such that the intracellular replication of the phage increases with bacterial growth rate. The cell volume was also measured using Feret's diameter which indicated an increase in cell volume for host cells grown in LB media and 60% LB, compared to 40%, 20% LB and 80 mM and 100 mM glucose in DMM. The nutrient concentration of growth media affects the number of phages produced due to a significant increase in growth rate. The positive correlation between growth rate and burst size for each of the growth media was statistically significant and nutrient concentration played a key role in the rate of growth of the bacterial host cell. The relationship between growth rate and mean cell volume indicates the effect of limiting nutrients on the number of phage progeny produced. Therefore, I've concluded that nutrient rich media such as LB have an increased growth rate and cell volume which results in an increased burst size.

Acknowledgements

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INTRODUCTION

1.1 Bacteriophage Lambda Genome and Structure

Bacteriophages—phages for short—are bacterial viruses that “eat” and destroy bacteria. Lambda phage was discovered in 1951 by Esther Lederberg who found that they were released from *Escherichia coli* bacteria by ultraviolet irradiation (Lederberg, 1951). Phage lambda is a *Siphovirus* with a flexible 150 nm noncontractile tail, an icosahedral capsid and a double stranded DNA genome (Abedon, 2008, Figure 1). The lambda phage tail is used to attach to maltose porin LamB on the cell surface of *E. coli*. LamB is composed of three identical subunits each formed by an 18-stranded antiparallel β -barrel which forms a channel through the outer membrane. Lambda phage uses this channel for ejecting its genetic material into bacteria, and once inside the cell, the double stranded linear DNA circularizes (Shatterjee and Rothenberg, 2012).

The lambda phages were one of the earliest model systems for the study of DNA and genes (Davidson and Szybalski, 1971). The lambda virion chromosome was also among the first natural linear DNAs which was found to lack blunt ends but to have complementary 12 bp protruding 5'-ends called cohesive (or sticky) ends, since the two single strand ends can pair and thus bind to each other (Hershey *et al.*, 1963; Hershey and Burgi, 1965).

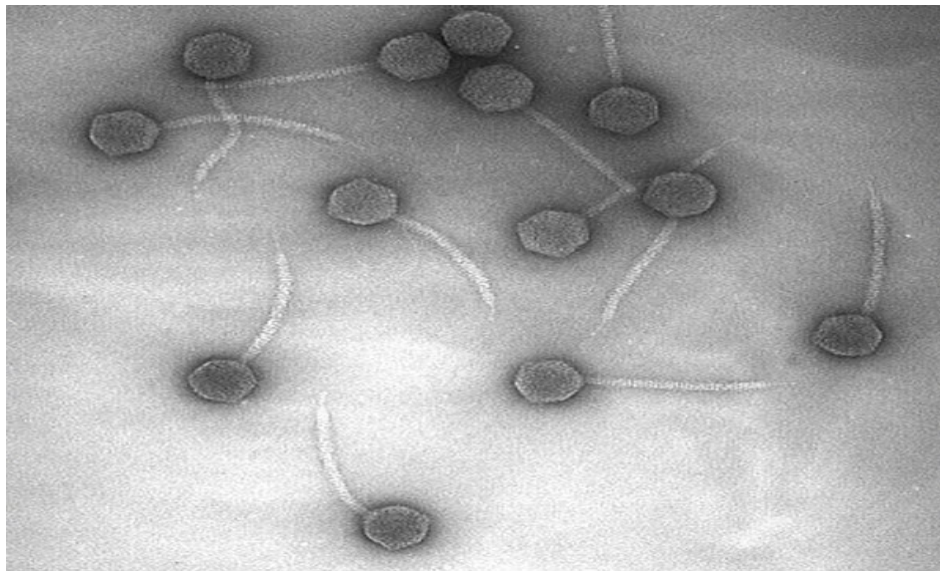
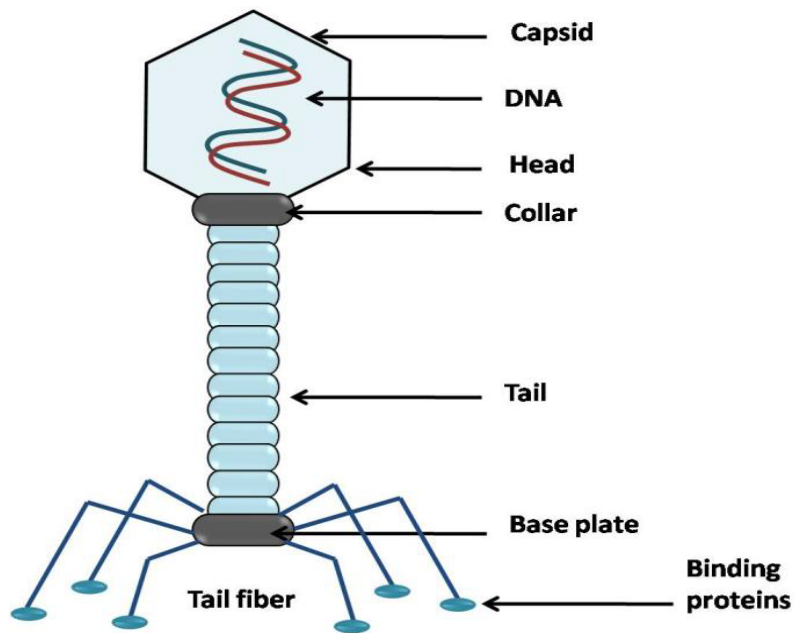


Figure 1. Lambda phage (phage.org)

1.2 Phage Life Cycle and Replication

The life cycle of a virus can be divided into three successive stages: (1) “searching” for a susceptible host cell to initiate an infection, (2) producing viral progeny inside the infected host, and (3) exiting current host cell to start a new infection cycle (Shao and Wang, 2008). From the

point of view of a lytic bacteriophage, the three stages of its life cycle correspond to the processes of adsorption, maturation, and lysis. The rates and timing of these processes can thus be seen as a phage's life-history traits (Shao and Wang, 2008).

Bacteriophage λ is a temperate phage and can enter the lytic or lysogenic cycle of replication upon infection of *E. coli* (Fogg et al., 2010). Once the *E. coli* bacterial cell is infected by bacteriophage lambda, a decision is made between cell death (lysis) and viral dormancy (lysogeny) (Ptashne, 2004), which is a process that serves as a simple paradigm for decision-making between alternative cell-fates during development (Court et al., 2006; St-Pierre and Endy, 2008). During the decision process, the regulatory circuit encoded by viral genes (primarily *cI*, *cII* and *cro*) integrates multiple physiological and environmental signals, including the number of infecting viruses and the metabolic state of the cell, in order to reach a decision (Weitz et al., 2008). The lysis-lysogeny decision characterizes the genetic developmental networks. First, after infection of the host bacterium, a decision between lytic or lysogenic development is made that is dependent upon environmental signals and the number of infecting phages per cell. Second, the lysogenic prophage state is very stable. Third, the prophage enters lytic development in response to DNA-damaging agents. The CI and Cro regulators define the lysogenic and lytic states, respectively, as a bistable genetic switch. Whereas CI maintains a stable lysogenic state, recent studies indicate that Cro sets the lytic course not by directly blocking CI expression but indirectly by lowering levels of CII which activates *cI* transcription (Oppenheim et al. 2005).

E. coli cells that are multiply infected tend to become lysogens whereas singly infected cells tend to be lysed. As shown in Figure 3, lysogenization takes place in cells infected with more than one phage (Kourilsky, 1973). Infection starts by the adsorption of phage particles to the *E. coli* cell surface receptor site (on the bacterial cell wall) and penetrates it to insert its DNA (Figure 2). Subsequently, DNA that is ejected into the cytoplasm circularizes immediately for replication and gene expression. In the decision-making stage, several factors can affect the infection outcome, such as gene expression, phage voting (infecting phages could each “vote,” or commit separately to decisions in single cells as if possessing “individuality”), and phage interactions. The spatial organization of phage gene products might also impact the infection outcome. At the final stage of infection, phages can lyse or lysogenize the cell. Phage confusion is also possible where phage DNA integration and cell lysis happens simultaneously (Shao et al., 2018).

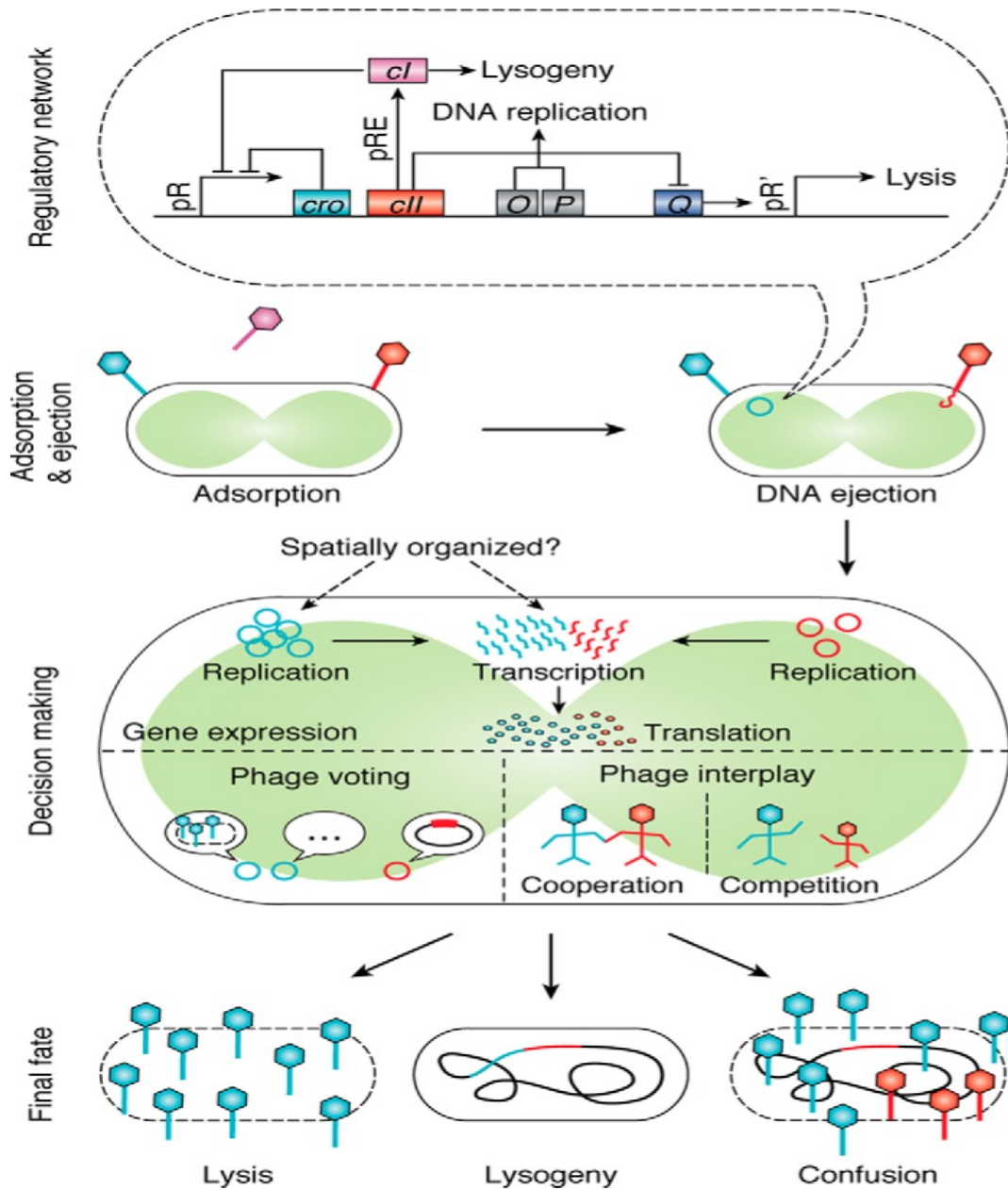


Figure 2. Key steps in lambda infection and decision making (Shao et al. 2018).

As mentioned, the decision to lyse a cell or enter lysogeny is stochastic. Cells that become lysogenic may later spontaneously induce, leading to virion production and cell lysis (Joh and Weitz, 2011). The lytic cycle goes from infection to production of progeny or virions and results in the release of new phages. The phage DNA directs the cell's metabolism to

produce viral components (phage DNA and protein). Empty phage heads are formed and packed with DNA in which the phage mature with the addition of collars, sheaths and base plates to the head along with tail fibers. Hence the lysis of the bacterial cell produces completed infective phages (Young, 2014; Young and Bläsi, 1995) and the host DNA is destroyed in this cycle (Fogg et al. 2010).

In the lysogenic (latent) cycle, the λ chromosome is integrated into the E. coli chromosome and is referred to as the prophage. Phage DNA is replicated along with the bacterial chromosome prior to cell division. Once the cell divides by binary fission each daughter cell carries the incorporated phage DNA (Semsey et al , 2015, Figure 3). The prophage can also be induced to leave the host DNA and initiate a lytic cycle (Casjens and Hendrix, 2015). A lysogen is considered stable unless it receives a signal and gets induced, in which all the lysogens switch to lytic propagation. In the lytic pathway as mentioned, the λ genome replicates free from the bacterial genome, directs the production of phage capsid proteins and the bacterial host is killed which releases about 100 new phage particles (Noreen and Gann, 2007).

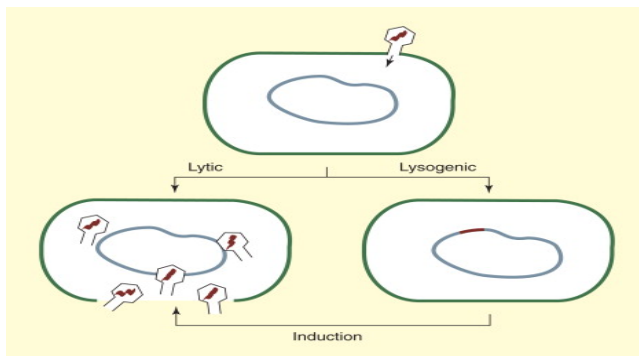
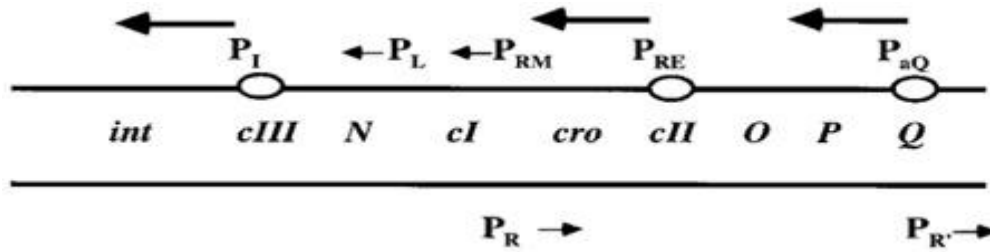


Figure 3. Life cycle of phage lambda (Noreen and Gann, 2007).

The genetic and molecular bases for many of these traits have been known for quite some time and the study of these processes has formed the foundation of modern molecular biology (Stent 1965). To initiate an infection, a phage virion has to first adsorb onto the surface of a susceptible host cell. This is accomplished by the recognition of receptors on the host cell surface by phage tail fiber (or attachment protein) and various other appendages (Katsura 1983; Goldberg et al. 1994). Only a few phage gene products, usually in the range of one to three, are involved in the adsorption process. The initial step of host recognition is commonly seen as a ligand-receptor binding problem; therefore, the rate of “finding” a host is usually assumed to follow the mass-action kinetics (Shao and Wang, 2008).

The choice between lysis and lysogeny is governed largely by the interactions of five regulatory proteins called CI, CII, Cro, N, and Q. These proteins regulate transcription from a number of promoters in a regulatory region of the phage DNA (Court et al. 2007, Figure 5). In bacteriophage λ the level of lambda protein CII determines the decision for the lytic-lysogenic cycle. Low levels of CII protein allow for CI production and the switch to the lysogenic cycle. Higher levels of CII protein results in lower levels of CI repressor and consequently a lytic cycle. (Zeng et al. 2010, Figure 4).



(Obuchowski et al, 1997)

Figure 4. Regulatory regions in phage lambda.The genes are shown between the lines. The leftward promoters p_L , p_{RM} , p_{RE} , p_I , and p_{aQ} and the rightward promoters p_R and $p_{R'}$ are shown (arrows). CII-activated promoters are indicated by bubbles, and the direction of their transcripts is shown by arrows.

The process of transcription and gene expression is a controlled pattern of immediate early, early (also called delayed early) and late transcription (Echols, 1971; Friedman and Gottesman, 1983). Factors, such as lower temperature, minimal media (less nutrient-rich media) or high multiplicities of phage infection, bias the lytic-lysogeny decision for phage lambda to favor lysogeny over the lytic cycle. The decision to switch is an example of how the environment can influence gene regulation during development. The state of the host cell is sensed by phage regulatory proteins and determines which pathway shall be taken. The regulatory sequence along each pathway is a cascade- group of genes are turned on and off sequentially (Ptashne, 2004).

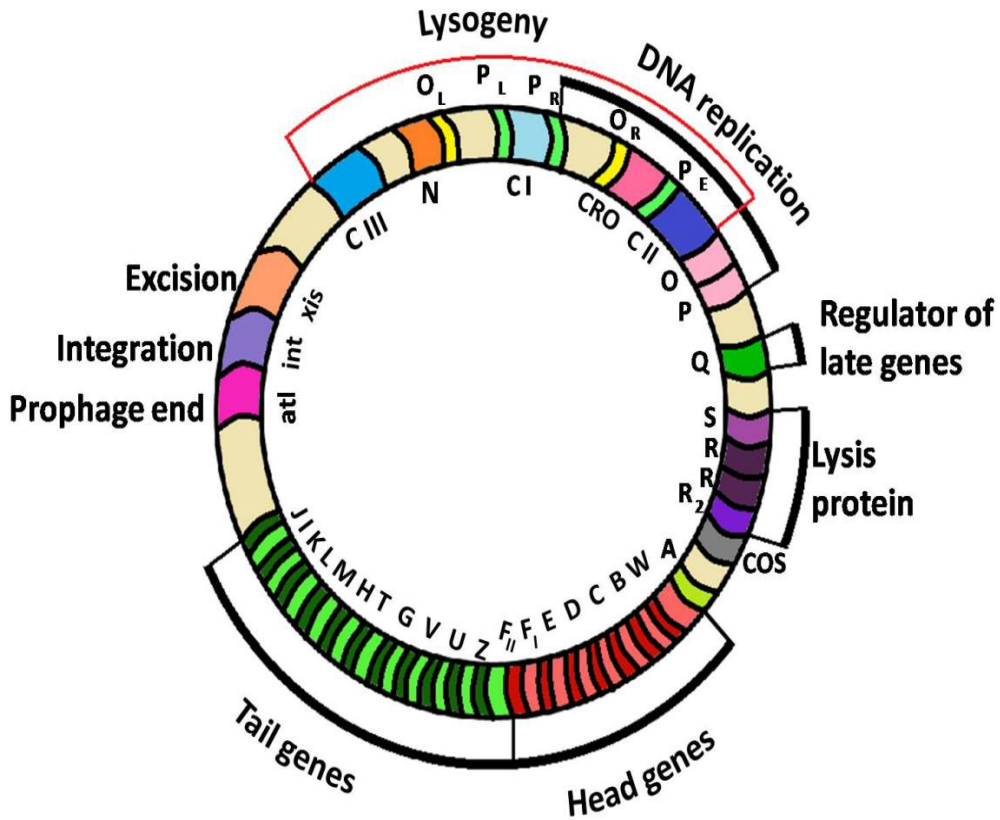


Figure 5. Genetic mapping of lambda phage

1.3 Phage Burst Size Determination

Phage growth parameters include phage rate of adsorption, latent period, eclipse period, and burst size (Hyman and Abedon, 2009). After infection of a host in an appropriate physiological state, new phage particles are synthesized, leading, in general, to the rapid lysis of the bacterial cell and the concomitant release of the newly formed virions. The multiplication rate of phages can be determined by two parameters: the burst size (the number of particles released in one cycle of infection) and the latency period (the time between infection and lysis of the host). The number of progeny produced per infected cell control rates of phage population growth and burst size is defined as the number of viruses released from each infected cell (Stent, 1963, Figure 6). Burst sizes for different viruses have a large range which corresponds to the

range of different sizes found in the host cells (Hyman, 2009). For example, SIV, a cousin and model for the HIV virus, is released from infected T cells with a burst size of $\approx 50,000$ whereas cyanobacterial viruses have characteristic burst sizes of $\approx 40-80$ and phage lambda and other phages (such as T4, T5 and T7) attacking bacteria have burst sizes of $\approx 100-300$ (Hyman, 2009).

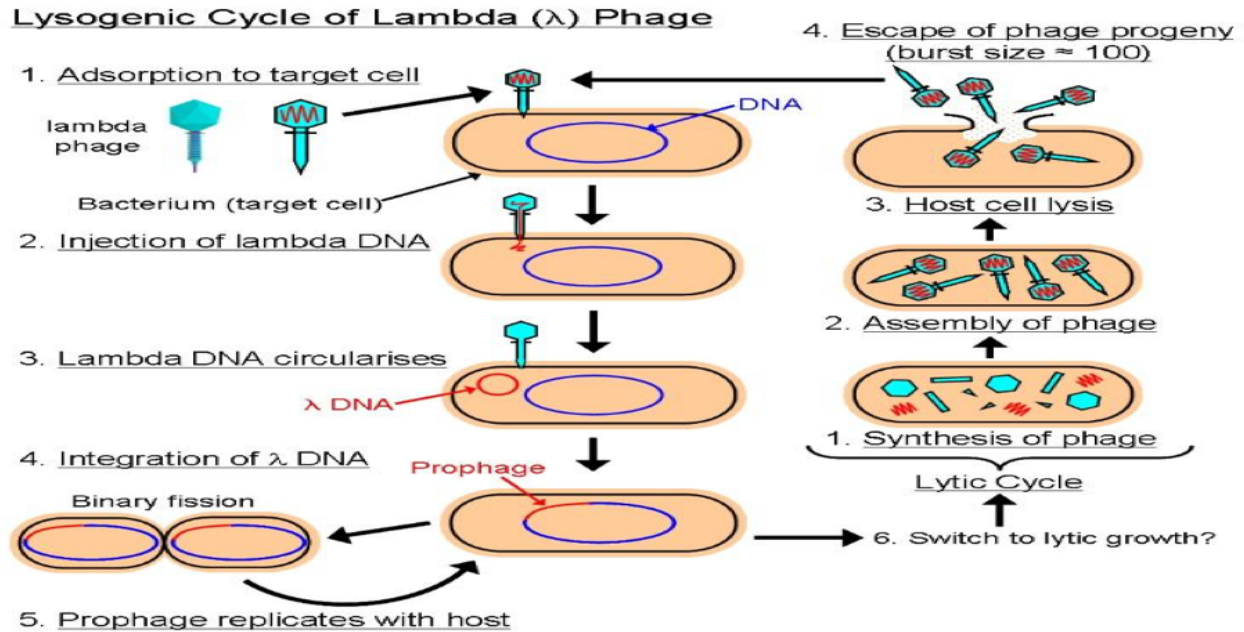


Figure 6: The cycle of lysogeny and lysis with release of virions known as burst size.

Phage λ lyses the host *Escherichia coli* at a precisely scheduled time after induction. Lysis timing is determined and controlled by the action of phage holins ('hole'-formers'), which are small proteins that induce hole formation in the bacterium's cytoplasmic membrane (Young, 1992; Young 2014; Young and Wang 2000). Holins are considered lysis time-keepers and the formation of the λ holin hole in the membrane is hypothesized to be a multi-step process that starts with the transcription of the late mRNA and the translation of the S holin protein. The resulting holin monomers are then inserted into the cell membrane, where they dimerize, then oligomerize, eventually leading to the formation of higher-order holin aggregates, or rafts, in the

cell membrane (Gründling et al., 2000, Figure 7). When considering the release of phage progeny, the lysis time is determined by when a critical holin concentration is reached in the cell membrane (Dennehy and Wang, 2011). When the phage components for virion assembly are produced, phage holins and endolysins are translated and create exits for the assembled phages. Phage holins had a certain role in the lysis delay, since these proteins need to be inserted into the bacterial membrane and accumulate to create a hole for the transport of endolysins for degrading the peptidoglycan cell wall (Kim et al., 2018).

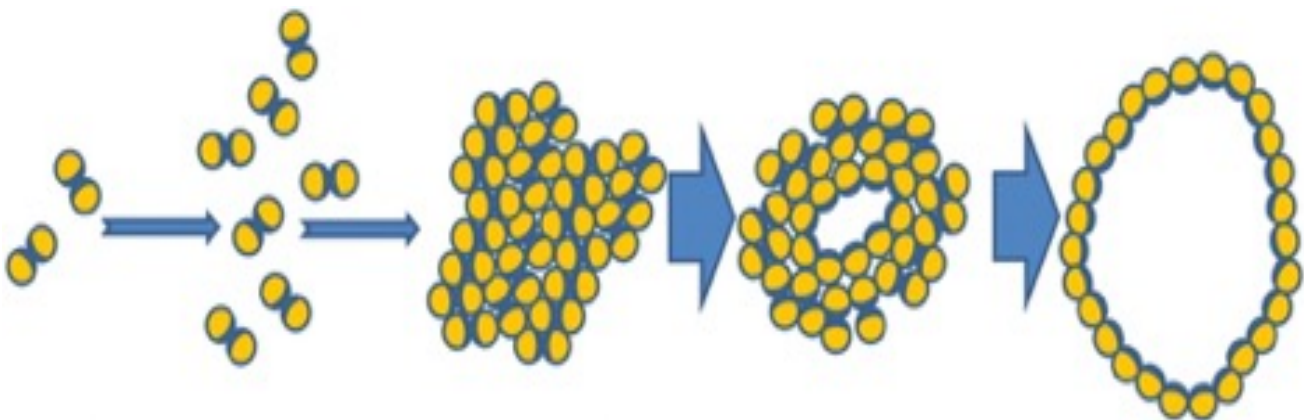


Figure 7: Holin accumulation and formation of a hole in the bacterial cell wall (Singh and Dennehy, 2014).

1.4 The role of nutrients on number of phages produced

Cells of *E. coli* that enter the human/animal stomach with ingested food are confronted with severe stress due to the low pH in the intestinal tract. Following stomach passage, they may then encounter conditions that are amenable to growth and survival in the intestinal system. In contrast, conditions in the open environment are very different (van Elsas et al. 2011). Thus, fluctuating but generally low concentrations of available carbon/energy sources, temperatures,

oxic, anoxic conditions and variable (low to high) osmolarity will be present. In the light of these challenging conditions, most forms of *E. coli* seem to have conserved particular key evolutionary adaptations in their core genomes, as indicated by the presence in most strains of import systems, such as siderophore-mediated iron uptake systems (Schubert et al, 2004) or ABC transporters for uptake of amino acids and sugars. This suggests a high ability in *E. coli* to obtain diverse nutrients, aiding in its survival in open environments (Ihssen et al., 2007).

On the other hand, growth and survival of *E. coli* in open environments is often restricted by the availability of nutrients and energy sources. In a growing culture, starvation will ensue at a given moment due to a limitation of particular carbon or other substrates. Under such starvation conditions, the cells progressively metabolize their cellular carbohydrates, followed by proteins and RNA, while initially protecting the DNA. In most open environments, *E. coli* may behave similarly when nutrients exhaust. However, it will first respond to the different—often complex—energy sources and organic compounds, which are usually present in low concentrations. Under such low-nutrient conditions, different alternative catabolic functions and binding proteins will become derepressed, as found in cultures under glucose or arabinose limitation (Ihssen and Egli, 2005). Similarly, iron may become limiting and thus iron-acquisition operons might also become derepressed. It would thus be interesting to test the environmental fitness and gene expression of the iron-acquisition-island-containing strains. Furthermore, *E. coli* was also found to exhibit a high degree of catabolic flexibility, which conferred a clear fitness advantage in its secondary habitats such as soil and water (Ihssen and Egli, 2005).

Nutrient availability is important in the regulation of size and growth rate in bacteria such as *E. Coli*. Cells can grow larger and faster in nutrient rich media compared to nutrient poor media (Yao et al. 2012). According to the Centers for Disease Control and Prevention (CDC) *E. Coli* are bacteria found in the environment, foods and intestines of humans and animals. The range of conditions for these bacteria include the nutrient rich gut of the intestines to nutrient poor environments such as soil, water and host environments such as macrophages (Peterson et al., 2005). Nutrient poor is defined as lacking essential building blocks for growth including carbon, nitrogen and phosphorus. 'Lysogeny' or Luria Broth (LB) is the commonly used growth medium for the host bacteria in experimental studies because it promotes fast growth (Lessard, 2013). In rich media bacteriophage lambda produces early proteins in excess amounts needed for effective propagation and replication proteins may be the limiting factors for phage lytic growth in poor media.

Glucose is also a nutrient media for *E. Coli* and cells which are cultured in very low concentrations of glucose (minimal media) impair the lytic development and produce fewer number of phages resulting in a lower burst size. Under favorable conditions (e.g LB growth media) phage λ produces excess proteins such that lytic growth can be achieved under unfavorable conditions. Proteins such as O and P decrease as the nutritional value of the media decreases and becomes limiting for phage growth (Gabig et al. 1998). When *E. Coli* is starved by limiting nutrient availability it goes through a stationary phase which protects the cell and promotes survival in harsh conditions (Peterson et al. 2005). It is also important to note that low nutrient levels results in a slower growth of the cell which in turn decreases the number of phage

progeny. Therefore, the growth rate of the host defines the number of phages that will be produced from the infected bacterium.

1.5 The Effect of Growth Media on Growth Rate of the Host and Concentration of Phages

The concentration of bacteria and bacterial physiological state have a significant influence on the number of phages produced per infected cell (Abedon et al., 2001; Golec, et al., 2014; Hadas et al., 1997; Middelboe, 2000; You et al., 2002). LB medium contains low amount of carbohydrates and other utilizable carbon sources such as peptides and free amino acids important for *E. coli* growth (Sezonov et al. 2007; Wang and Koch, 1978) but also low amount of divalent cations (Ca^{2+} and Mg^{2+}) (Wee and Wilkinson, 1988). Catabolizable amino acids are clearly plentiful in Luria-Bertani broth, presumably in the form of oligopeptides. It has been reported that wild-type *E. coli* K-12 growing in tryptone broth sequentially catabolizes l-serine, l-aspartate, l-tryptophan, l-glutamate, glycine, l-threonine, and l-alanine. It may follow the same order, although one would also expect l-proline, a good carbon source for *E. coli*, to be depleted early on. At later times, l-arginine, l-glutamine, l-asparagine, l-cysteine, and l-lysine, for all of which *E. coli* possesses catabolic systems, may be consumed as well (Sezonov et al. 2007). If the concentration of nutrients in a medium such as LB is decreased, it affects the growth rate of the bacterial cell which in turn can influence the burst size. When limiting nutrient concentration (glucose, phosphate or tryptophan) the host growth rate decreases which reduces the burst size (Shehata and Marr, 1971).

In *Escherichia coli*, the number of ribosomes in the cell is proportional to the growth rate in order to meet the demand for protein synthesis (Nomura *et al.*, 1984; Bremer and Dennis, 1996; Keener and Nomura, 1996). Understanding the intracellular processes of bacterial cells helps to determine internal cell regulation. Phages depend on the intracellular resources of their

hosts, which further depends on the physiological state of their hosts. As the growth rate of the host increases, so does its cell size (You et al., 2002), as well as its intracellular levels of genomic DNA, RNA polymerase, ribosomes, nucleoside triphosphates (NTPs), and amino acids. These are all essential resources that affect phage growth. In addition, the effects of these factors may be convoluted: the increase in the cell volume as the host cell grows faster will affect the concentrations of various resources, which in turn may affect phage growth (You et al., 2002).

OBJECTIVES

In my research experiment I determined the phage lambda burst size in *E. Coli* grown in different concentrations of nutrient media including LB, 60 % LB, 40% LB, 20% LB, 80 mM and 100 mM glucose in DMM. The bacterial cell volume was also measured to investigate the relationship between cell size and the number of phages produced in each of the growth conditions mentioned. The aim of the study was to understand how phage (virus) assembly is controlled based on the physiological state of the host bacterial cell. The growth rate influences the burst size in phage lambda which is also linked to the cell volume. Faster growing cells have more replicative machinery therefore they have a larger burst size compared to slow growing cells. I also wanted to observe the nutrient media dependent growth rate of the bacterial cells on virus replication as a way to reveal the significance of cellular regulation and internal molecular processes of cells. My experiment reveals the importance of growth rate of the bacterial host cell as a factor which affects phage lambda gene expression and replication.

MATERIALS AND METHODS

Bacterial and Phage Strains. *E. coli* MC4100 (ID JJD 2, Source CGSC#6152) and bacteriophage λ cI857 S_{WT} was chosen to study bacteriophage infections of *E. coli* (ID JJD3).

Growth Conditions and Cell culture. An overnight culture of *E. coli* JJD3 (JJD2 + prophage) was grown in different formulations of media LB: 60% LB (6 g Bacto[®] tryptone [Becton, Dickinson and Co., Sparks, MD], 3 g yeast and 10 g NaCl per L ddH₂O), 40% LB (4 g Bacto[®] tryptone [Becton, Dickinson and Co., Sparks, MD], 2 g yeast and 10 g NaCl per L ddH₂O), 20 % LB (2 g Bacto[®] tryptone, 1 g yeast and 10 g NaCl per L ddH₂O), 80 mM glucose in Davis minimal salts (7.0 g K₂HPO₄, 2.0 g KH₂PO₄, 0.5 g Sodium citrate . 2H₂O, 0.2 g MgSO₄ . 7H₂O, 1.0 g (NH₄)₂SO₄) and 100 mM glucose in Davis minimal salts. A single bacterial colony was inoculated from an agar plate into 10 mL of the respective media in a sterile flask and placed on a shaker (200 rpm) inside a 35°C incubator. To achieve an OD₆₀₀ between 0.2-0.3, 500 µL of the overnight culture was added to 30 mL respective media (exponential media) and incubated at 37°C until the OD₆₀₀ ~ 0.3. At 20-minute increments up to 2 hours, a turbidity reading was taken using a spectrophotometer set to 600 nm. Growth rates were calculated as the slope of the linear regression of the natural logarithm transformed OD₆₀₀ values over time (in minutes).

After reaching an OD₆₀₀ ~ 0.3, the bacterial colony sample was added by serial dilution to M9 salts and 100 µL of the dilutions were plated by spread plate procedure. LB agar plates were incubated at 30°C overnight incubator and bacterial colonies were counted after 16-18 hours.

Phage Titer Determination. Phage style mixing was used to mix each of the serial dilutions. Three milliliters of LB with 0.7% agar (w/v) (H top agar (tryptone (10.0 g), sodium chloride (8.0 g), agar (480 g), distilled water (up to 1 L), pH > 6) was melted in a microwave and maintained in a liquefied state in a 50°C water bath before adding to the bacterial culture and plating) was mixed with 400 µl of overnight bacterial culture and then poured on LB agar plate with 1.4%

agar (w/v). Triplicates of the lysates were added to the overnight JJD2 exponential culture and then added to 3 ml of H top agar by phage style mixing. LB agar plates were incubated at 37°C overnight and plaques were enumerated after approximately 16–18 hr of incubation.

Phage Stock Preparation. Chloroform was added to the exponential growth media (about 10% of remaining media) and let stand for 15 minutes at room temperature to check the number of unattached phages. Five milliliters of the lysate was transferred via a sterile syringe and centrifuged at 8,000 rpm for 10 minutes. Tenfold serial dilutions up to 10^{-9} of the prepared λ bacteriophage stock solution were prepared. Dilutions of phage samples were prepared in SM buffer (1 g gelatin, 5.8 g NaCl, 2 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 50 ml 1 mol/L Tris-HCl (pH 7.5), distilled water to 1 L) and mixed with *E. Coli* (300 microliters JJD2 + 100 microliters of dilution) and let stand for 15 minutes. Subsequently, 3.0 ml of H top agar was mixed with 400 μl of overnight bacterial culture and then poured on LB agar plate with 1.4% agar (w/v) which was left to solidify for 10 minutes. LB agar plates were incubated at 37°C overnight and plaques were enumerated after approximately 16–18 hr of incubation.

Burst Size Determination. The concentration of phages ($\text{PFU} \cdot \text{ml}^{-1}$) was determined using standard double agar overlay plaque assay (Kropinski, Mazzocco, Waddell, Lingohr, & Johnson, 2009). Burst size was calculated by dividing the number of phages formed by the number of bacterial colonies formed before induction ($\text{PFU} \cdot \text{ml}^{-1}$) / ($\text{CFU} \cdot \text{ml}^{-1}$).

Cell volume measurement

About 5 μl of cell culture at ($\text{OD}_{600} = 0.3\text{--}0.4$) was applied to a cover slip and covered with a 2-mm-thick layer of 2% agar in order to immobilize the cells and hold them flat to the cover slip.

Cells were imaged using a 60× phase contrast objective (NA 1.40) with an Olympus inverted microscope and camera. All image analysis was performed using the ImageJ suite of tools. Phase contrast images were captured immediately after sampling from exponential phase culture ($OD_{600} = 0.3\text{--}0.4$). Cell size was obtained by tracing the whole individual cell and not including the background. Next, the “Feret’s diameter” was calculated for each cell, giving both the longest (length, L) and shortest (width, W) caliper distance along the boundary of the selected area. Bacteria were considered as cylinders with hemispherical ends at each side. Bacterial length was calculated by means of a Feret box enclosing the object measured at different angles. The maximal Feret’s diameter (fe_{\max}) at a certain angle equals the length of the object, and the minimal Feret’s diameter represents the width (W) of the object. Cell volume (V) was calculated according to the equation $V = \pi R^2 * (L - 2R/3)$. Bacterial length (L) was defined as fe_{\max} and the minimal Feret’s diameter represents the width used for calculation of the radius ($R = W/2$) of the objects. If fe_{\max} is less than $length_{AP}$, bacterial length is described by $length_{AP}$ and bacterial width is described as area divided by $length_{AP}$. For each growth condition, 100 individual cells were measured.

Statistical Analysis

Pearson’s Goodness of Fit in linear regression analyses were used to examine the relationship between burst size and growth rate (min.^{-1}). The burst size per unit of volume was not normally distributed. For the normalized burst size measurements, the burst size of each condition per cell volume of the bacterial cell host was transformed to the log scale in order to normalize the data points (Figure 10). A one way ANOVA was used to interpret the statistical

significance for the cell volume measurements for the six growth media (LB, 60% LB , 40% LB, 20 % LB, 80 mM and 100 mM glucose in DMM).

RESULTS

In this study the burst size of phage lambda was calculated using growth conditions in varying concentrations of LB and glucose in DMM media. In general cells with higher growth rates lead to higher burst size counts since fast growing cells have more machinery (ribosomes, resources), which can produce phage faster and leads to a higher number of phage progeny (burst size) (You et al., 2002). The growth rate of the bacteria was calculated for each of the growth conditions using the change in OD₆₀₀ readings over time to determine the bacterial rate of growth per min. Results indicated a positive linear correlation between the burst size and growth rate ($R^2= 0.71$, $p < 0.05$) and this suggests that burst size increases with the increase in *E. Coli* growth rate (Figure 8).

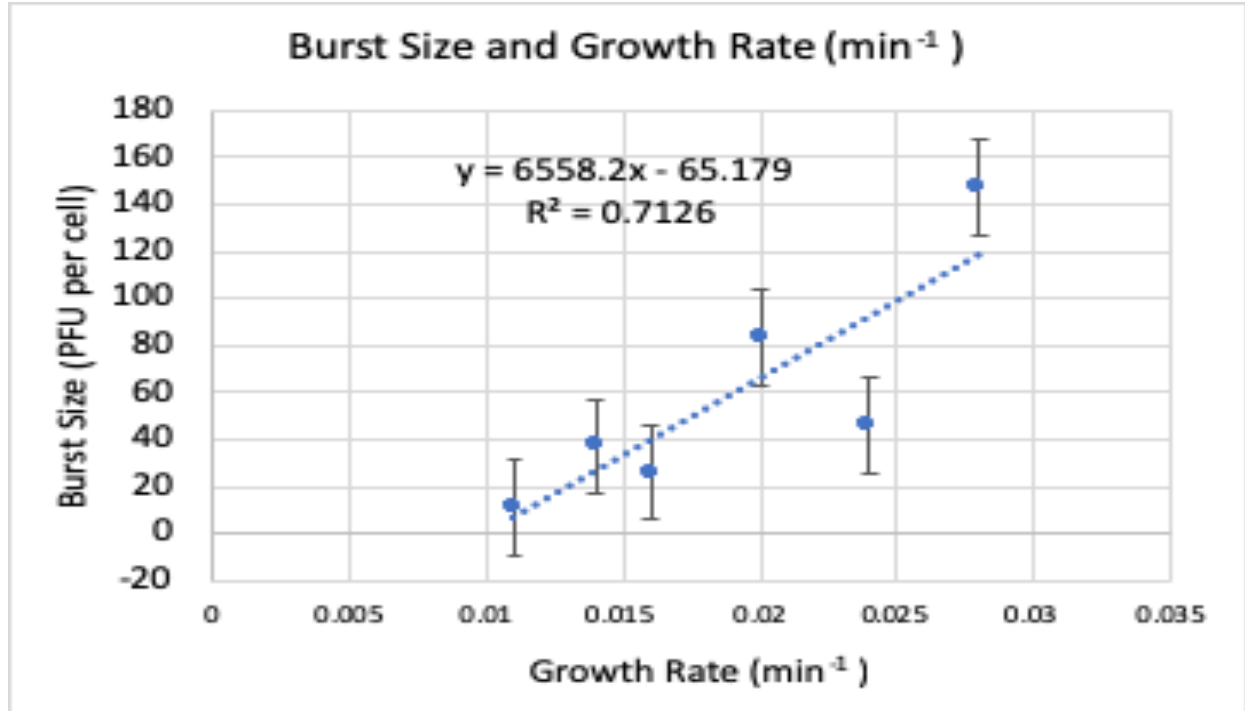


Figure 8: Goodness of fit linear regression of growth rate (min.⁻¹) and burst size. Blue dots with error bars represent experimental data, while dotted lines represent best fit.

The growth rate of the host bacterial cell significantly changed when the nutrient level of the media was reduced and resulted in slower growth of the cell which resulted in a lower number of phage particles released from the cell. Linear regression on log-transformed data for normalized burst size (analysis of log transformed data as a normal distribution of my results) was determined to understand the relationship between growth rate (as a life history trait) and normalized burst size per unit of volume. For normalized burst size the constant number of phages per unit of volume was determined. Volume is proportional to the number of phages produced in the bacterial cell. Therefore, cell volume is proportional to the number of phages

produced and as volume increases the burst size also increases. The results showed that normalized burst size also had a positive linear correlation with growth rate ($R^2= 0.64$, $p < 0.001$) and significantly changes based on the growth media used in the experiment (Figure 9).

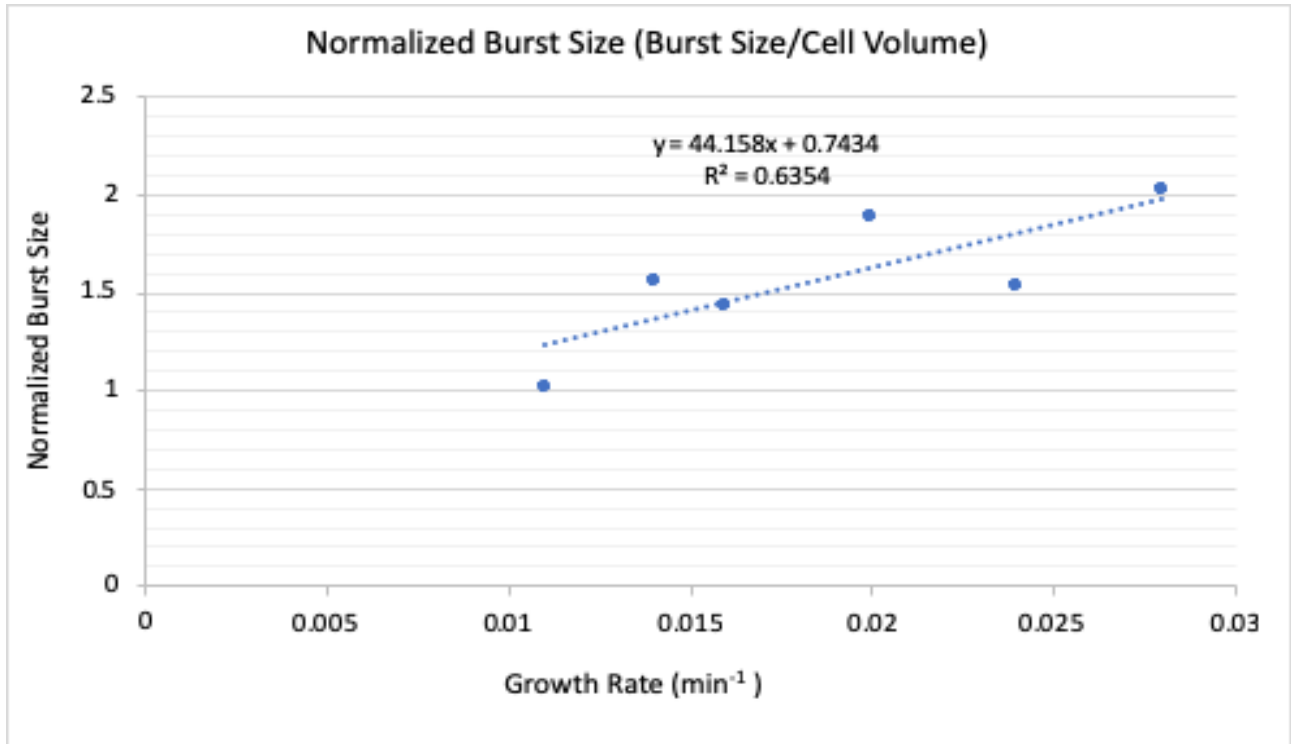


Figure 9: Correlation between normalized burst size and cell volume with growth rate (min.⁻¹).

Since the expression of the phage holin gene is entirely dependent on the host, a lower host growth rate would lead to a lower rate of holin protein synthesis, thus resulting in a longer lysis time. For my results, lysis time was measured as the time after thermal induction and lysis of the cell with respect to the growth rate. In comparison to the burst size the lysis time decreases with increasing rate of growth and doesn't have a positive linear relationship with burst size (Figure 10). It is concluded that phage progeny accumulates and lysis time has no effect on the burst size. Higher growth rate produces more progeny but at the same time the burst size increase is

not positively linked to lysis time. The lysis time decreases as the burst size increases with increasing growth rate when comparing Figure 8 and 10.

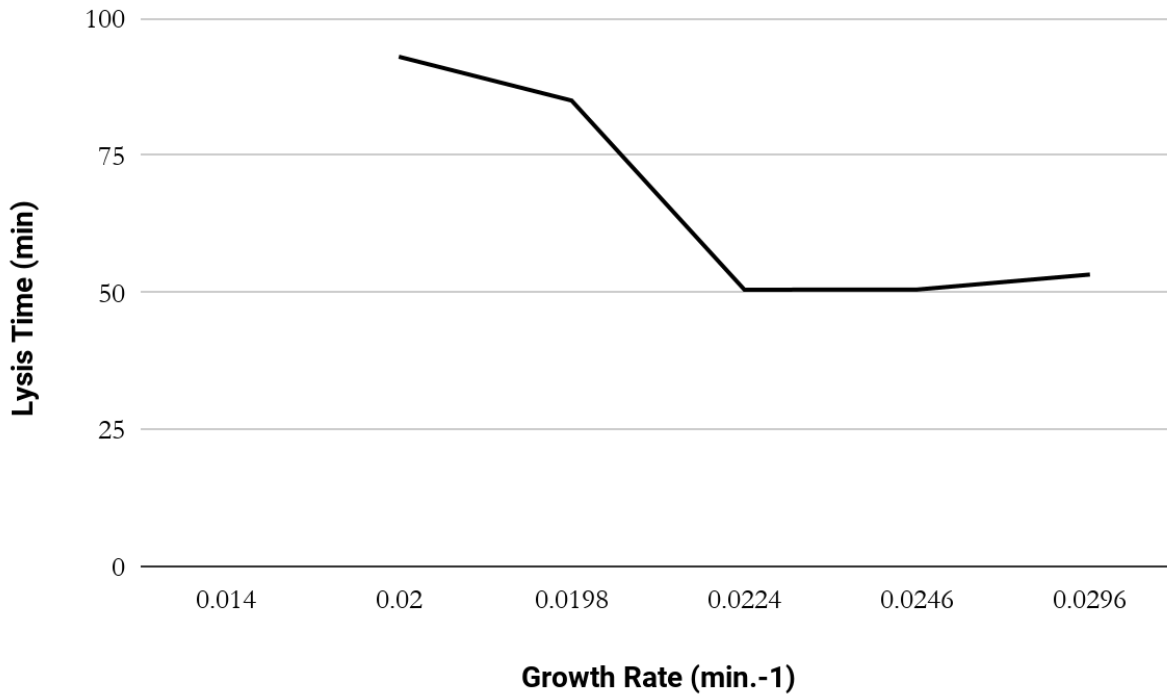


Figure 10: Lysis time and growth rate.

Using a microscope-mounted, temperature-controlled perfusion chamber, the cell volume was observed and measured for *Escherichia coli* lysogens (exponential media). These observations revealed a considerable amount of variation in cell volume measurements in the bacterial cells when grown in varying levels of LB media and glucose in DMM. A one-way analysis of variance (ANOVA) was conducted to compare the effect of growth media (LB, 60% LB , 40% LB, 20 % LB, 80 mM and 100 mM glucose in DMM) on cell volume measurements (Table 1). There was a significant effect of growth media on cell volume of the host cell at the $p < .05$ level for the six conditions as determined by one-way ANOVA ($F(5, 594) = 17.1427, p < .001$) (Table 3). As presented in Table 2, post hoc comparisons using the Tukey HSD test indicated

that the mean score for LB ($M = 1.38$, $SD = 0.47$) and 60% LB ($M = 1.28$, $SD = 0.39$), was significantly different than the 40% LB ($M = 0.95$, $SD = 0.35$), 20% LB ($M = 1.04$, $SD = 0.41$), 100 mM ($M = 1.05$, $SD = 0.42$), and 80 mM ($M = 1.02$, $SD = 0.39$) conditions. Specifically, our results suggest that cell volume varied significantly when grown in nutrient poor media compared to nutrient rich media such as LB and 60% LB, therefore growth conditions are a significant factor in host cell volume measurements (Figure 11).

Table 1

ANOVA Comparisons of Cell Volume in Growth Media Conditions

Growth Condition	n	Mean (10^{-9} μ L)	SD
LB	100	1.381	0.47
60% LB	100	1.283	0.39
40% LB	100	0.955	0.35
20% LB	100	1.040	0.41
100 mM	100	1.054	0.42
80 mM	100	1.016	0.39

Table 2

Comparison	Tukey HSD inference (Mi-Mj)	Tukey HSD Q statistic	Tukey HSD p-value
LB-40% LB	p<0.01	2.4029	0.0010053
LB-20% LB	p<0.01	10.4169	0.0010053
LB-100 mM	p<0.01	8.3368	0.0010053
LB-80 mM	p<0.01	8.0018	0.0010053
60% LB-40%	p<0.01	8.9165	0.0010053
60% LB-20%	p<0.01	8.0140	0.0010053
60% LB-100 mM	p<0.01	5.9340	0.0011811
60% LB-80 mM	p<0.01	5.5989	0.0010053

Table 3*Summary of ANOVA*

	Sum of Squares	df	Mean Square	F	p-value
Between Groups	14.34	5	2.86	17.14	0
Within Groups	99.41	594	0.1674		
Total	113.76	599			

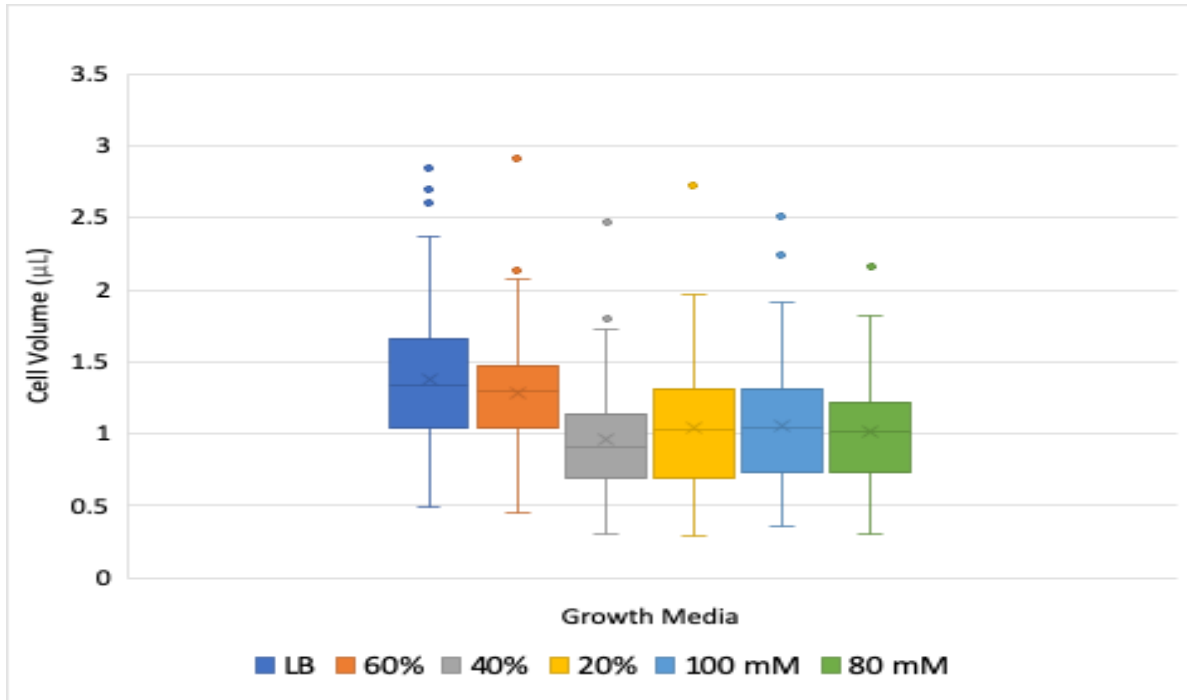


Figure 11: Cell volume measurements (10^{-9} μL) in growth media was highest in LB and 60% LB media but was significantly different from the 40% LB, 20% LB, 100 mM and 80 mM (glucose in DMM) cell volume size.

There was a significant difference in growth rate (min.^{-1}) among the different growth media based on the nutrient resource. The growth rate was lowest in the 20% LB and highest in LB (Figure 12). Since the growth rate is positively correlated with burst size it suggests that there will be a higher burst size in rich media such as LB, 60% LB and 100 mM glucose in DMM.

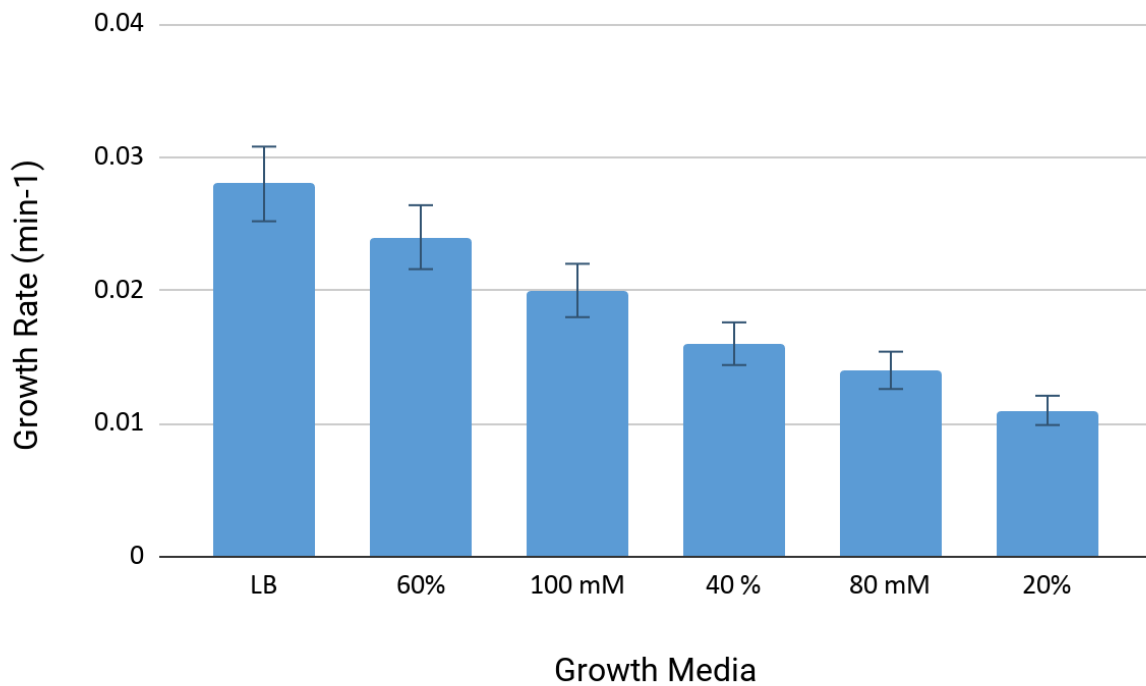


Figure 12: Growth rate in the six different growth media.

I observed that cell growth rate was directly affected by the nutrient content of the growth media, therefore the cellular machinery of the host cell and phage depend on the growth conditions. This leads to a higher rate of holin protein synthesis and an increase in the number of phage progeny released from the host cell.

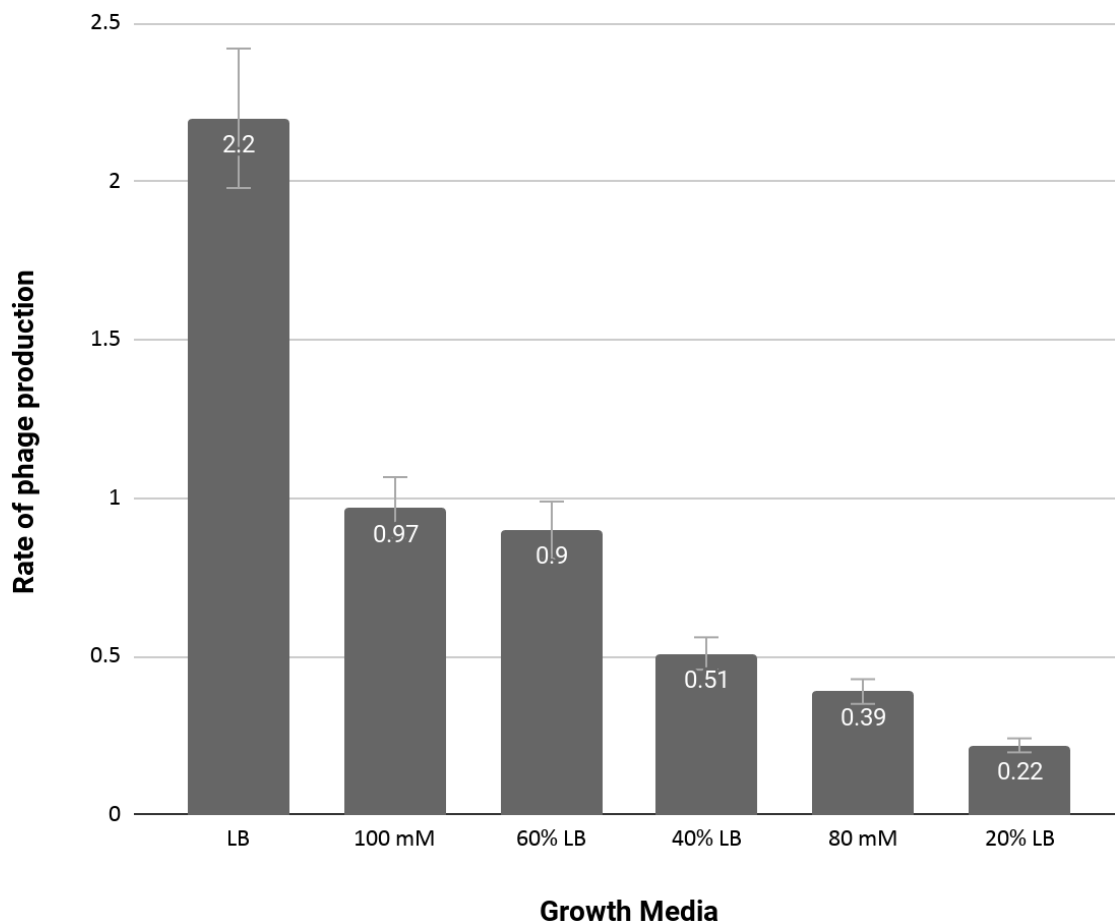


Figure 13: Rate of phage production (burst size/lysis time) (min.⁻¹) in varying growth media.

Since different media presented different growth rates for the bacterial cell host the rate of phage production was measured as the number of phages produced per min. Results revealed that phage burst size was highest in LB media and decreases as the nutrient concentration level decreases, in which the 20 % LB had the lowest amount of phage progeny and 100 mM glucose was a better media when compared to 40 % LB (Figure 13).

DISCUSSION

Bacterial cells are simpler models which are commonly used to understand more complex cells. The regulation of internal processes such as holin production is a way to compensate for the change in physiological state of the host cell such as growth rate. Results show that at low nutrient concentrations the growth rate of the bacterial host cell *E. coli* decreases (Figure 12). Nutrient rich media such as LB increases the growth rate of the bacterial cells and produces a higher number of phage progeny. The growth media of the host cell was varied based on the concentration of LB medium including 60% LB, 40% LB, 20% LB and 80 mM and 100 mM glucose in DMM. LB medium, which is complex, possesses limitations due to low amount of carbohydrates and other utilizable carbon sources such as peptides and free amino acids therefore it is important for *E. coli* growth (Sezonov et al., 2007; Wang and Koch, 1978). Glucose in DMM (minimal medium) contains the necessary nutrients for the growth of wild type *Escherichia coli* strains and dextrose is the available energy source for the cells. Dipotassium and monopotassium phosphates provide buffering to the medium. Magnesium sulphate and ammonium sulphate are sources of ions that stimulate metabolism (Davis, 1949).

Based on my data, burst size increased with the increase in growth rate of the host cell. The growth rate in LB media and 60 % LB was found to be the highest compared to the other dilutions and correlated positively with burst size. The lowest burst size was for 20% LB which also had the lowest growth rate compared to the other growth media. Higher burst size counts depend on the rate of growth of the host cell which varies based on the nutritional content of the media. Host bacterial cell growth affects the burst size for phage lambda depending on the nutrient level available (Figure 8).

In addition, the cell volume according to the equation $V = \pi R^2 * (L - 2R/3)$, for the bacterial cells was also determined. Based on the measurements, the mean cell volume for the given growth conditions between groups was significantly different when compared to LB and 60 % LB. The 80 mM glucose in DMM growth media had the smallest cell volume. Higher nutrient availability promoted higher growth rate and an increase in the cell volume (Figure 11). Therefore, LB and 60% LB are considered the ideal nutrient rich media for bacterial cells which can influence the speed of intracellular phage replication. Cell volume also increases in faster growing cells therefore cell size increases with increasing growth rate. When considering phage progeny an increase in cell volume is related to a faster rate of growth which results in an increased number of phage particles released from the cell. As a result, host cell growth rate and the burst size have a positive linear relationship and high nutrient media promotes rapid growing cells.

The availability of glucose in the minimal media was also observed to produce a higher number of phages when compared to the 20% LB media due to the higher nutrient concentration. Even though LB is an ideal growth media for *E. Coli*, glucose in DMM provided a better growth medium when the concentration of LB was reduced. The burst size comparison of 80 mM and 100 mM glucose in DMM was also different such that a higher phage production was observed in 100 mM. But as noted the amount of nutrient availability to the host cell affects the growth rate and results in significant changes in phage production (Figure 13).

When considering phage production as a unit of time, the rate was highest in LB media which proves the significance of nutrient media in the production of phage particles at a given lysis time (Figure 13). Studies by Wang et al., had also shown that bacteriophage fitness (growth rate) had a positive linear relationship with lysis timing such that burst size increased when the

lysis time also increased (2006). In my study, lysis timing and burst size did not have a linear relationship such that burst size increases with increasing growth rate, but lysis time decreases with increasing growth rate. Figure 13 reveals phage production rate (min^{-1}) which was determined using the lysis timing based on the burst size and it can be concluded that LB had more than a double fold of phage production when compared to the other growth media. As the nutrient level was lowered the number of phages produced also decreased and this indicates that the growth of bacterial cells depends on the rich media which speeds up viral replication.

According to Nabergoj et al. (2018), burst size increased linearly with an increase in growth rate of phage T4 in rich medium (LB), and this was also observed in this study for phage lambda. The results in my study clearly showed that bacterial growth rate has an important influence on phage lambda growth parameters such as burst size. When the dilution of LB medium was decreased the burst size also increased (Figure 8), therefore it changed in a similar fashion as it has been already described in the literature (Abedon et al., 2001; Golec et al., 2014; Hadas et al., 1997). It is interesting that burst size linearly ($R^2 = .71$) increased with increasing growth rate in the studied growth media (Figure 8). My study also used dilutions of glucose in Davis minimal salts to observe the growth rate compared to LB. The availability of glucose in higher concentrations provided a better medium for *E. Coli* growth in comparison to LB dilutions of 40 % and 20%.

When considering lysis time and burst size it has been shown in previous studies by Wang (2006) that there is a positive linear relationship in λ -phages, such that the longer the lysis time is, the larger the burst size. In my study the lysis time decreases with increasing growth rate of the bacterial cell and there is no linear relationship between burst size and lysis time as was

expected. Therefore, lysis time has no effect on burst size in my study and phage progeny accumulates but lysis time decreases with increasing growth rate (Figure 10).

Previous studies by Clark et al. had observed the contrast in phage production depending on the growth media used for cell growth. Growth in complex medium showed a higher burst size compared to defined medium (Clark et al. 1986). In my study the burst size also increased when the growth rate of the bacterial cell was high which was dependent on the nutrient level of the growth media (Figure 9). This further concludes the observation that cell volume also plays a role in explaining the reason for the higher number of phage progeny. Overall metabolic constraints affect the fitness and rate of growth of the bacterial cell and I observe a relationship between burst size and growth rate which confirm that the variation in phage production and replication depends on the host cell growth.

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